The interaction between St John's wort and an oral contraceptive

Objectives: **The popular herbal remedy St John's wort is an inducer of cytochrome P450 (CYP) 3A enzymes and may reduce the efficacy of oral contraceptives. Therefore we evaluated the effect of St John's wort on the disposition and efficacy of Ortho-Novum 1/35 (Ortho-McNeil Pharmaceutical, Inc, Raritan, NJ), a popular combination oral contraceptive pill containing ethinyl estradiol (INN, ethinylestradiol) and norethindrone (INN, norethisterone).**

Methods: **Twelve healthy premenopausal women who were using oral contraception (>3 months) received a combination oral contraceptive pill (Ortho-Novum 1/35) for 3 consecutive 28-day menstrual cycles. During the second and third cycles, the participants received 300 mg St John's wort 3 times a day. The serum concentrations of ethinyl estradiol (day 7), norethindrone (day 7), follicle-stimulating hormone (days 12-16), luteinizing hormone (days 12-16), progesterone (day 21), and intravenous and oral midazolam (days 22 and 23) were determined in serial blood samples. The incidence of breakthrough bleeding was quantified during the first and third cycles.**

Results: **Concomitant use of St John's wort was associated with a significant ($P < .05$) increase in the oral clearance of norethindrone (8.2 ± 2.7 L/h to 9.5 ± 3.4 L/h, $P = .042$) and a significant reduction in the half-life of ethinyl estradiol (23.4 ± 19.5 hours to 12.2 ± 7.1 hours, $P = .023$). The oral clearance of midazolam was significantly increased (109.2 ± 47.9 L/h to 166.7 ± 81.3 L/h, $P = .007$) during St John's wort administration, but the systemic clearance of midazolam was unchanged (37.7 ± 11.3 L/h to 39.0 ± 10.3 L/h, $P = .567$). Serum concentrations of follicle-stimulating hormone, luteinizing hormone, and progesterone were not significantly affected by St John's wort dosing ($P > .05$). Breakthrough bleeding occurred in 2 of 12 women in the control phase compared with 7 of 12 women in the St John's wort phase. The oral clearance of midazolam after St John's wort dosing was greater in women who had breakthrough bleeding (215.9 ± 66.5 L/h) than in those who did not (97.5 ± 37.2 L/h) ($P = .005$).**

Conclusion: **St John's wort causes an induction of ethinyl estradiol–norethindrone metabolism consistent with increased CYP3A activity. Women taking oral contraceptive pills should be counseled to expect breakthrough bleeding and should consider adding a barrier method of contraception when consuming St Johns wort. (Clin Pharmacol Ther 2003;74:525-35.)**

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St John's wort (*Hypericum perforatum*) is a popular herbal medicine marketed as a dietary supplement and widely used for the treatment of mild to moderate depression.[1] St John's wort was reported to have efficacy comparable to that of the selective serotonin reuptake inhibitors and tricyclic antidepressants for the treatment of mild to moderate depression, as well as a better side effect profile.[2,3] St John's wort enjoys wide-

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spread use in the United States and Europe, with 106 million daily doses of *Hypericum* extract taken in Germany alone.[4] Although St John's wort appears to be well tolerated in the general population, the potential for unsupervised self-medication by individuals in an effort to enhance their mood provides the opportunity for numerous drug–dietary supplement interactions. Indeed, the use of St John's wort in combination with prescribed medication has been associated with numerous reports of clinically relevant adverse drug interactions.[5]

In organ transplant recipients, concomitant use of cyclosporine (INN, ciclosporin) and St John's wort has been associated with subtherapeutic levels of cyclosporine, leading to acute graft rejection.[6,7] In healthy volunteers, when St John's wort was coadministered with indinavir, a 57% reduction in the indinavir area under the concentration versus time curve from 0 to 8 hours was observed.[8] These changes in pharmacokinetic characteristics are consistent with induction of synthesis of cytochrome P450 (CYP) 3A enzymes and the membrane transporter P-glycoprotein in the liver and the intestinal wall. Hyperforin, a major constituent of St John's wort, binds to the human pregnane X receptor and increases the transcription rate of *CYP3A4* and *MDR1*.[9,10]

Drugs that induce CYP3A enzymes, such as rifampin (INN, rifampicin), have been associated with reduced efficacy or failure of oral contraceptives.[11] This interaction is thought to reflect the significant contribution of hepatic and intestinal wall CYP3A enzymes to the oxidative metabolism of 17α-ethinyl estradiol.[12,13] CYP3A enzymes and possibly others that are involved in the oxidation of norethindrone (INN, norethisterone) are also induced by rifampin and rifabutin.[14] Recently, there have been case reports of breakthrough bleeding in individuals who were stabilized by use of ethinyl estradiol (INN, ethinylestradiol)–containing oral contraceptives after coadministration of St John's wort.[15] In addition, there is speculation by the lay press that St John's wort consumption is associated with the birth of "miracle babies."[16,17] Schwarz et al[18] have recently reported the loss of oral contraceptive efficacy after St John's wort coadministration in 4 women; this failure of contraception resulted in the termination of unwanted pregnancies.

Despite the reasonable expectation, on the basis of the data described here, that St John's wort, as an inducer of CYP3A4, may reduce the efficacy of oral contraceptives, this proposition has not yet been addressed in a controlled clinical study. The objectives of this study were to evaluate the effect of St John's wort on oral contraceptive efficacy with suppression of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and incidence of breakthrough bleeding used as end points and to assess the effect of St John's wort on the disposition of ethinyl estradiol and norethindrone. In view of the uncertain identity of all of the active constituents of St John's wort and their presence in a given preparation, we also quantified the effect of St John's wort on hepatic and intestinal CYP3A activity by using intravenous and oral midazolam.

METHODS

Subjects. After approval by the Institutional Review Board of Indiana University–Purdue University Indianapolis and the Research Involving Human Subjects Committee of the Food and Drug Administration, 12 healthy nonsmoking women aged 27 ± 7 years (weight, 65 ± 10 kg) gave written informed consent to participate. Volunteers were eligible if they had been taking combination oral contraceptive pills for at least 3 months before the study without adverse events, such as breakthrough bleeding. During the study, the participants were requested to use a second reliable method of contraception, and urine pregnancy tests were conducted at the beginning of each study cycle. The exclusion criteria were a significant medical history, history of any localized or systemic infectious disease within 4 weeks before admission, and use of prescription drugs (other than oral contraceptive pills) or over-the-counter medications. We also excluded subjects with a history of or current alcohol or drug abuse, blood donation within the past 2 months, use of tobacco in any form, or use of St John's wort within 4 weeks of the study, as well as allergy to St John's wort or midazolam.

Between 1 and 6 weeks before enrollment into the study, all subjects underwent a thorough medical examination, including a complete blood cell count with differential and platelet counts; urine pregnancy test; serum chemical 17-parameter analysis; analysis of triglyceride, lactate dehydrogenase, alanine transaminase, and γ-glutamyl transpeptidase levels; and urinalysis with microscopy. For at least 2 weeks before the start of the study and until the end of the study, volunteers were requested not to eat any food or drink any beverage containing grapefruit or grapefruit juice. During the first and third months of the study, the volunteers abstained from ethanol. In addition, food or beverage items containing xanthines (eg, coffee, tea, Mountain Dew soft drink [Pepsi-Cola North America, Purchase, NY], chocolate, cola) were stopped 48 hours before and

during the pharmacokinetic study periods. On the first day of the study, all of the blood and urine screening clinical parameters were repeated in the volunteers. Vital signs and weight were obtained throughout the study.

Study design. The volunteers kept a daily diary throughout the study in which they recorded the occurrence and severity of headaches, cramps, menstrual flow, and breakthrough bleeding. Breakthrough bleeding was defined as one or more occurrences of uterine bleeding between menstrual periods. No information that identified the likelihood or time course of these possible side effects was provided to the subjects. Telephone contact was made regularly to reinforce the requirement for keeping the diary. We conducted a fixed-order study, over three 28-day menstrual cycles (Fig 1). In the first cycle (month 1) the volunteers received Ortho-Novum 1/35 (Ortho-McNeil Pharmaceutical, Inc, Raritan, NJ) every morning for 21 days and placebo on days 22 through 28. During the second and third cycles (months 2 and 3), in addition to the oral contraceptive pills, the volunteers received St John's wort extract (Rexall-Sundown Pharmaceuticals, Boca Raton, Fla), 300 mg 3 times a day with food from day 1 through day 28.

On the seventh day of the first and third cycles, volunteers were admitted to the General Clinical Research Center (GCRC) for 24 hours for determination of the serum concentration–time curve of ethinyl estradiol and norethindrone (and hyperforin in month 3 only) by use of an intravenous catheter inserted in a forearm vein for withdrawal of blood samples. Volunteers received a light breakfast and a single 300-mg dose of St John's wort (month 3 only), and then a predose blood sample (10 mL) was collected. One hour after the breakfast, a single dose of Ortho-Novum 1/35 was administered with 240 mL of tap water. Blood samples (10 mL) were obtained at 15, 30, 45, 60, 75, and 90 minutes and at 2, 3, 4, 8, 12, and 24 hours after dosing. The subjects were then discharged from the GCRC after 24 hours and following a light breakfast. The subjects reported to the GCRC after 48 hours to return the urine collection. On days 12 through 16 of the first and third cycles, volunteers returned to the GCRC and a blood sample (12 mL) was obtained before the dose of oral contraceptive was administered for quantitation of serum concentrations of progesterone, FSH, and LH. In addition, the serum concentration of progesterone was determined on day 21 of the first and third cycles.

Finally, during the first and third cycles (days 21 and 22), volunteers were admitted to the GCRC for 48 hours for quantification of the pharmacokinetics of intravenous and oral midazolam. After a light breakfast, volunteers emptied the bladder and a blood sample was collected (15 mL, predose sample plus serum progesterone). One hour after breakfast, a 0.05-mg/kg dose of midazolam (Versed; Roche Laboratories, Nutley, NJ) was infused intravenously over a 30-minute period into a brachial vein. Blood samples (5 mL) were collected from a contralateral brachial vein at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours after intravenous midazolam. Urine was collected over 12-hour intervals for 24 hours for the determination of parent drug and metabolite concentrations. Twenty-four hours after the administration of the intravenous midazolam, an oral dose of midazolam, 5 mg, was administered with 240 mL of tap water. Blood samples (5 mL) were collected from an indwelling catheter at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 hours after drug administration. The baseline blood sample for the oral dose was the 24-hour blood sample from the intravenous dose.

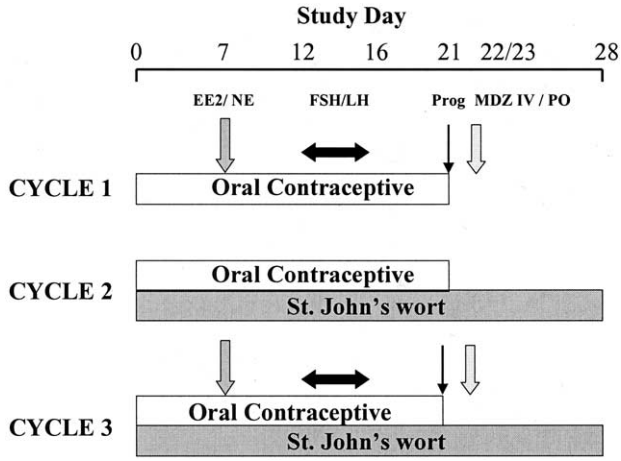

Fig 1. Schedule of study drugs and sampling points: Ethinyl estradiol (EE2) and norethindrone (NE) serum concentration–time curves were obtained on day 7. Serum concentration–time curves for intravenous (IV) and oral (PO) midazolam were obtained on days 22 and 23, respectively. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) serum concentrations were obtained on days 12 through 16. Serum progesterone (Prog) concentrations were determined on day 21.

Sample analysis. Serum and urine concentrations of midazolam, 1'-hydroxymidazolam, and 4-hydroxymidazolam were quantified by HPLC via mass spectrometric detection as described previously.[19] Serum and capsule concentrations of hyperforin, hypericin, and

pseudohypericin derived from St John's wort extract were analyzed by HPLC with mass spectrometric detection as previously described.[20] Serum concentrations of FSH and LH were quantified by use of a chemiluminescent assay (Automated Chemiluminescence System; Chiro Diagnostics Corp, Emeryville, Calif); detection limits were 0.5 mIU/mL for LH and 0.3 mIU/mL for FSH. Plasma progesterone was quantified by use of a competitive immunoassay with a detection limit of 0.05 ng/mL (AIA-PACK; Tosoh Medics Inc, San Francisco, Calif).

Serum concentrations of ethinyl estradiol were determined by gas chromatography–mass spectrometry (Anapharm, Montreal, Quebec, Canada). In brief, ethinyl estradiol and internal standard (2α-testosterone, 1 ng) were extracted from 0.50 mL of serum by use of silica solid-phase extraction cartridges, eluted with 3 mL of hexane/ethyl acetate (50:50). The eluate was dried under vacuum (TurboVap LV; Zymark Corporation, Hopkinton, Mass), reconstituted with pentafluorobenzoyl chloride/ethyl acetate (1:10), heated at 70°C (Reacti-therm III; CellPro, Inc, Bothell, Wash), evaporated to dryness, and reconstituted with 0.50 mL of sodium bicarbonate (0.5 mol/L). Hexane was added (3 mL), and the supernatant was transferred and evaporated. The residue was reconstituted with 50 μL of silylation reagent and injected into a Hewlett-Packard 6890 series gas chromatograph (Hewlett-Packard, Palo Alto, Calif) interfaced to a mass spectrometer (Hewlett-Packard 5973). Peaks of interest were separated by use of a DB-17 column (Agilent Technologies, Palo Alto, Calif) and a carrier gas of helium flowing at a rate of 1.3 mL^{-1}. The mass spectrometer was run in negative chemical ionization mode with methane as collision gas and column, source, and interface temperatures of 150°C, 270°C, and 300°C, respectively. Ethinyl estradiol and 2α-testosterone (internal standard) were quantified with selective ion monitoring at 562 mass-to-charge ratio (*m/z*) and 566 *m/z*, respectively. The retention times of ethinyl estradiol and internal standard were 8.89 and 8.85 minutes, respectively, and the limit of quantitation was 5.0 pg/mL. Values from 3 of 4 pairs of duplicate samples were within 5% of the mean value, with the final pair of duplicates within 15% of the mean value. No interference from the administration of St John's wort was detected

Serum concentrations of norethindrone were determined via HPLC with mass spectrometric detection. Norethindrone and 2α-testosterone (20 ng, internal standard) were extracted from 1 mL of serum by use of 3.5 mL of a 70:30 mixture of hexane and ethyl acetate. The organic layer was dried under vacuum (Speed-Vac concentrator; Thermo Savant, Milford, Mass). The residue was reconstituted with 200 μL of mobile phase (10-mmol/L ammonium acetate [pH 7]/methanol; 20:80 [vol/vol]) and injected into a Hewlett-Packard 1100 series HPLC system interfaced to a mass spectrometer (Navigator; Thermo Finnigan, San Jose, Calif). Peaks of interest were separated by use of a Luna cyano column (5 μm × 4.6 mm internal diameter × 250 mm; Phenomenex, Torrance, Calif). The effluent was delivered at 1 mL/min to the mass spectrometer, which was equipped with an atmospheric pressure chemical ionization probe, which was run in the positive ion mode with source and probe temperatures of 200°C and 550°C, respectively. The corona pin and the cone voltages were 5 kV and 20 V, respectively. Norethindrone and 2α-testosterone were quantified with selective ion monitoring at 299.2 *m/z* and 305.5 *m/z*, respectively. The retention times of norethindrone and 2α-testosterone were 3.3 and 3.0 minutes, respectively, and the limit of quantitation was 0.25 ng/mL. No interference from the administration of St John's wort was detected.

Pharmacokinetic analysis. Standard model-independent methods were used to determine the pharmacokinetic parameters of interest for midazolam, norethindrone, and ethinyl estradiol (WinNonlin, version 4.0; Pharsight, Mountain View, Calif). The area under the blood (midazolam) or serum (norethindrone and ethinyl estradiol) concentration versus time curve (AUC) was calculated with the use of a combination of trapezoidal and log-trapezoidal methods up to the last data point, followed by extrapolation to infinity by use of the terminal elimination rate constant. The terminal elimination rate constant was calculated by using the slope of the terminal log-linear decline in blood concentrations. The terminal half-life was calculated by dividing 0.693 by the terminal elimination rate constant. The serum concentrations of midazolam were converted into blood concentrations as previously described.[21] The clearance of midazolam, norethindrone, and ethinyl estradiol was determined as the administered dose divided by the AUC with extrapolation to infinity. The oral bioavailability of midazolam was determined from the ratio of the dose-normalized AUCs obtained from oral and intravenous administration. The hepatic availability and the availability across the intestinal wall for midazolam were determined from the hepatic extraction ratio and total bioavailability as previously described.[21] In light of the extensive and equivalent recovery of 1'-hydroxymidazolam for oral and intravenous dosing (Table I), the fraction of the dose absorbed (F_{ABS}) is assumed to be unity.[21]

Table I. Effect of St John's wort administration (300 mg 3 times a day for 8 weeks) on disposition of midazolam after intravenous and oral dosing in 12 healthy female volunteers using oral contraception (Ortho-Novum 1/35)

	Control	*St John's wort*	*P value*	*Ratio and 90% CI**
Intravenous				
AUC (0–∞) ([μg · h]/L)	93.2 ± 28.2	87.5 ± 17.5	.394	96% (86% to 105%)
CL_{IV} (L/h)	37.7 ± 11.3	39.0 ± 10.3	.567	105% (93% to 116%)
Half-life (h)	3.9 ± 1.1	3.5 ± 1.1	.056	88% (80% to 96%)
Vd_{SS} (L/kg)	3.1 ± 0.8	2.9 ± 0.9	.389	92% (82% to 103%)
Oral				
AUC (0–∞) ([μg · h]/L)	68.3 ± 74.2	40.0 ± 27.4	.076	65% (49% to 81%)
CL_{oral} (L/h)	109.2 ± 47.9	166.7 ± 81.3†	.007	145% (125% to 166%)
Half-life (h)	2.7 ± 0.6	2.0 ± 0.6†	.004	74% (62% to 85%)
C_{max} (μg/L)	14.0 ± 9.5	11.1 ± 4.0	.214	84% (63% to 106%)
t_{max} (h)	1.0 ± 0.6	1.0 ± 0.4	.704	115% (72% to 158%)
F_{oral}	0.43 ± 0.28	0.28 ± 0.15†	.011	72% (60% to 84%)
F_H	0.63 ± 0.09	0.62 ± 0.08	.574	98% (92% to 104%)
F_G	0.67 ± 0.34	0.46 ± 0.21†	.031	74% (60% to 87%)
Urinary recovery (%)				
Intravenous dose	77.4 ± 8.3	76.8 ± 16.0	.979	99% (88% to 111%)
Oral dose	77.9 ± 7.6	68.7 ± 14.2	.163	112% (100% to 123%)

CI, Confidence interval; AUC (0–∞), area under concentration versus time curve from time 0 to infinity; CL_{IV}, intravenous clearance; Vd_{SS}, volume of distribution at steady state; CL_{oral}, oral clearance; C_{max}, maximum concentration; t_{max}, time to maximum concentration; F_{oral}, oral bioavailability; F_H, hepatic availability; F_G, gut wall availability.

*Geometric mean ratio (×100%) of St John's wort/control and 90% CI of ratio.

†Significant difference determined by ANOVA ($P \leq .05$).

Statistics. Data are reported as mean ± SD. The data generated from pharmacokinetic analyses were analyzed with ANOVA and a paired Student *t* test as appropriate with the use of the JMP software program (version 5.0.1; SAS Institute, Cary, NC), and $P \leq .05$ was regarded as statistically significant.

The serum concentration of progesterone under baseline conditions was 1.06 ± 0.34 ng/mL, and therefore a sample size of 12 should allow the discrimination of a 33% difference between control and treatment values at $P < .05$ with a power of greater than 80%. Ninety percent confidence intervals for the geometric mean ratio of treatment phase over control phase were also used to evaluate the equivalence of systemic exposure in the presence and absence of St John's wort. A lack of interaction, when exposure-response relationships are not well understood or demonstrated, can be concluded if these 90% confidence intervals fall within the range of 80% to 125%.

RESULTS

All subjects completed the study, and no adverse events were attributed to study participation. On the basis of diary entries, compliance with oral contraceptive dosing was 100% and compliance with St John's wort dosing was at least 90%, with 10 of 12 individuals reporting greater than 98% compliance during month 3. Individuals reporting missed doses did not miss more than 1 dose in a 24-hour period. The St John's wort preparation (Rexall-Sundown Pharmaceuticals) was chosen because it is a market leader in the United States and was labeled to contain 0.3% hypericin. Our analysis determined the hypericin and hyperforin content in 10 randomly selected St John's wort capsules to be 1.1 ± 0.1 mg and 8.9 ± 0.4 mg, respectively. After long-term St John's wort therapy (300 mg 3 times daily for 8 weeks), the mean peak and trough concentrations of hyperforin were 31.1 ± 14.6 ng/mL and 16.3 ± 8.1 ng/mL, respectively, and the 24-hour AUC was 522 ± 251 ng · h/mL. Fig 2, *A*, shows the time course of mean hyperforin concentrations over a 24-hour period after the dose administered on day 7 of cycle 3.

The mean serum concentration–time curve for norethindrone and ethinyl estradiol on day 7 after oral administration before and after 8 weeks of St John's wort administration is shown in Fig 3. St John's wort administration was associated with significantly increased ($P = .042$) oral clearance (CL_{oral}) of norethindrone, from 8.2 ± 2.65 L/h to 9.5 ± 3.43 L/h (Table II). In addition, the peak concentration of norethindrone was significantly decreased ($P = .045$), from 17.4 ± 5.1 ng/mL to 16.4 ± 5.2 ng/mL, but there was no change in half-life or time to maximum serum concentration (Table II). The half-life of ethinyl estradiol was

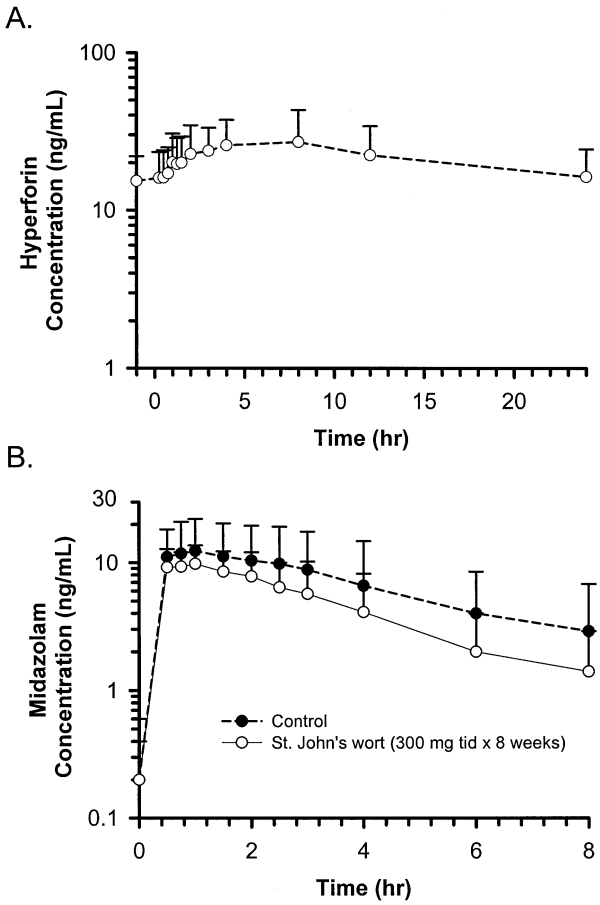

Fig 2. **A**, Mean (±SD) time course of serum hyperforin serum concentrations in 12 volunteers after oral administration of St John's wort (300 mg 3 times daily for 5 weeks) on study day 7 of cycle 3. **B**, Mean (±SD) midazolam blood concentrations after oral dosing on study day 22 during cycles 1 and 3 in 12 subjects. *Solid circles* represent mean (±SD) serum concentration before St John's wort administration, and *open circles* represent mean (±SD) after St John's wort administration.

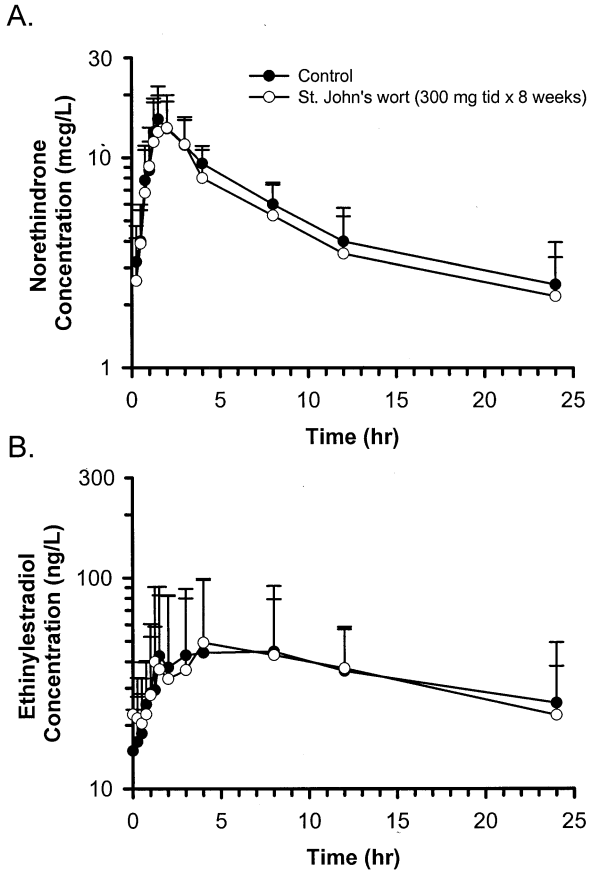

Fig 3. Effect of St John's wort (300 mg 3 times daily for 8 weeks) on disposition of norethindrone (**A**) and ethinyl estradiol (**B**) on study day 7 during cycles 1 and 3 (n = 12). *Solid circles* represent geometric mean (±SD) serum concentration before St John's wort administration, and *open circles* represent geometric mean (±SD) after St John's wort administration.

significantly reduced ($P = .023$) in the presence of St John's wort (from 23.4 ± 19.5 hours to 12.2 ± 7.1 hours), but there was no significant change in CL_{oral}, maximum serum concentration, or time to maximum serum concentration for ethinyl estradiol (Table II). The geometric mean ratio of the AUC of norethindrone to ethinyl estradiol was not significantly ($P \geq .05$) affected by St John's wort dosing (81 ± 106 versus 104 ± 496).

To examine whether the oral contraceptive–mediated suppression of peaks in the serum concentrations of FSH and LH was affected by St John's wort, we determined the ratio of the peak concentration to the mean serum concentration in each individual. St John's wort had no significant effect on ratios of the mean peak serum concentration to the mean serum concentration for FSH (Fig 4, *A*) and LH (Fig 4, *B*) at mid–menstrual cycle (days 12-16), but 6 of 12 volunteers had an increase in peak-to-mean ratio. The concentration of progesterone on day 21 (Fig 4, *C*) was unaffected by St John's wort dosing. On the other hand, 7 subjects reported breakthrough bleeding during long-term St John's wort therapy (cycle 3) compared with only 2 occurrences during the control phase (cycle 1; Fig 4, *D*). On the basis of the diary entries, the duration and quantity of menstrual flow and the frequency of head-

Table II. Effect of St John's wort administration (300 mg 3 times a day for 8 weeks) on disposition of norethindrone and ethinyl estradiol in 12 healthy female volunteers using oral contraception (Ortho-Novum 1/35)

	Control	*St John's wort*	*P value*	*Ratio and 90% CI**
Norethindrone				
AUC (0–24) ([μg · h]/L)	131.8 ± 35.1	118.3 ± 41.1	.150	88% (76% to 100%)
CL_{oral} (L/h)	8.2 ± 2.7	9.5 ± 3.4†	.042	114% (100% to 127%)
Half-life (h)	12.6 ± 7.2	12.1 ± 4.9	.799	102% (85% to 120%)
C_{max} (μg/L)	17.4 ± 5.1	16.4 ± 5.2†	.045	93% (88% to 98%)
t_{max} (h)	1.8 ± 0.8	1.7 ± 0.5	.614	96% (76% to 117%)
Ethinyl estradiol				
AUC (0–24) ([ng · h]/L)	2177 ± 1543	1661 ± 1324	.162	68% (14% to 123%)
CL_{oral} (L/h)	63.3 ± 71.6	93.1 ± 127.9	.411	85% (57% to 114%)
Half-life (h)	23.4 ± 19.5	12.2 ± 7.1†	.023	62% (15% to 110%)
C_{max} (ng/L)	97.3 ± 74.6	103.6 ± 78.9	.809	111% (33% to 189%)
t_{max} (h)	2.7 ± 2.9	2.2 ± 1.5	.131	63% (29% to 99%)

AUC (0–24), Area under serum concentration–time curve from 0 to 24 hours.
*Geometric mean ratio (×100%) of St John's wort/control and 90% CI of ratio.
†Significant difference determined by paired *t* test.

aches and cramps were unaffected by St John's wort therapy.

The effect of 8 weeks of St John's wort dosing on the mean serum concentration–time profile of oral midazolam is shown in Fig 2, *B*. After oral dosing, the clearance of midazolam was significantly ($P = .007$) increased by St John's wort, from 109 ± 48 L/h to 167 ± 8 L/h, and the half-life was significantly ($P = .004$) reduced, from 2.7 ± 0.6 hours to 2.0 ± 0.6 hours (Table I). The maximum midazolam blood concentration, time to maximum concentration, volume of distribution, and systemic clearance of midazolam were unaffected by St John's wort (Table I). The F_{oral} and F_G of midazolam were significantly reduced ($P < .05$) by St John's wort administration, whereas the F_H was unaffected (Table I).

Women who had breakthrough bleeding had a significantly higher ($P = .005$) oral midazolam clearance on day 23 of cycle 3, as compared with those who did not have breakthrough bleeding (215.9 ± 66.5 L/h versus 97.5 ± 37.2 L/h) (Fig 5). There was no difference in the CL_{oral} of midazolam at baseline between the 2 groups of women. The extent of induction (clearance with St John's wort therapy [CL_{SJW}] minus control clearance [$CL_{control}$]) was significantly greater ($P = .016$) in women who had breakthrough bleeding (88 ± 48 L/h) than in those who did not (11 ± 43 L/h). The percent change in midazolam CL_{oral} was not significantly different between women who had breakthrough bleeding (71% ± 38%) and those who did not (26% ± 40%) ($P = .08$). There was no significant difference ($P \geq .1$) in ethinyl estradiol exposure, norethindrone exposure, or the norethindrone–to–ethinyl estradiol ratio of the AUCs between women who had breakthrough bleeding and those who did not. No significant relationships were identified between the pharmacokinetic parameters for midazolam and norethindrone or ethinyl estradiol. The extent of norethindrone CL_{oral} induction was not significantly different ($P = .0596$) between women who did not have breakthrough bleeding (0.99 ± 0.20 L/h) and those who did (1.31 ± 0.29 L/h). There was no relationship between hyperforin exposure (maximum serum concentration, AUC) or progesterone serum concentration and the CL_{oral} of midazolam, the extent of CL_{oral} induction, or the occurrence of breakthrough bleeding.

DISCUSSION

In this study we evaluated the effect of SJW on the pharmacokinetics and efficacy of Ortho-Novum 1/35, an oral contraceptive pill containing norethindrone (1 mg) and ethinyl estradiol (0.035 mg) in 12 healthy young women. Importantly, St John's wort administration (300 mg 3 times daily for 8 weeks) to 12 women using oral contraception resulted in a significantly greater incidence of breakthrough bleeding. Breakthrough bleeding in oral contraceptive users is a major cause of discontinuation of hormonal contraceptives and substantially increases the risk of pregnancy as individuals switch to less reliable forms of contraception.[22,23] The precise pathogenesis of breakthrough bleeding in the face of hormonal contraception is unclear. In one report it was suggested that patients with breakthrough bleeding had ethinyl estradiol levels lower than 29 pg/mL; however, other reports have suggested an association with an abnormal estrogen/

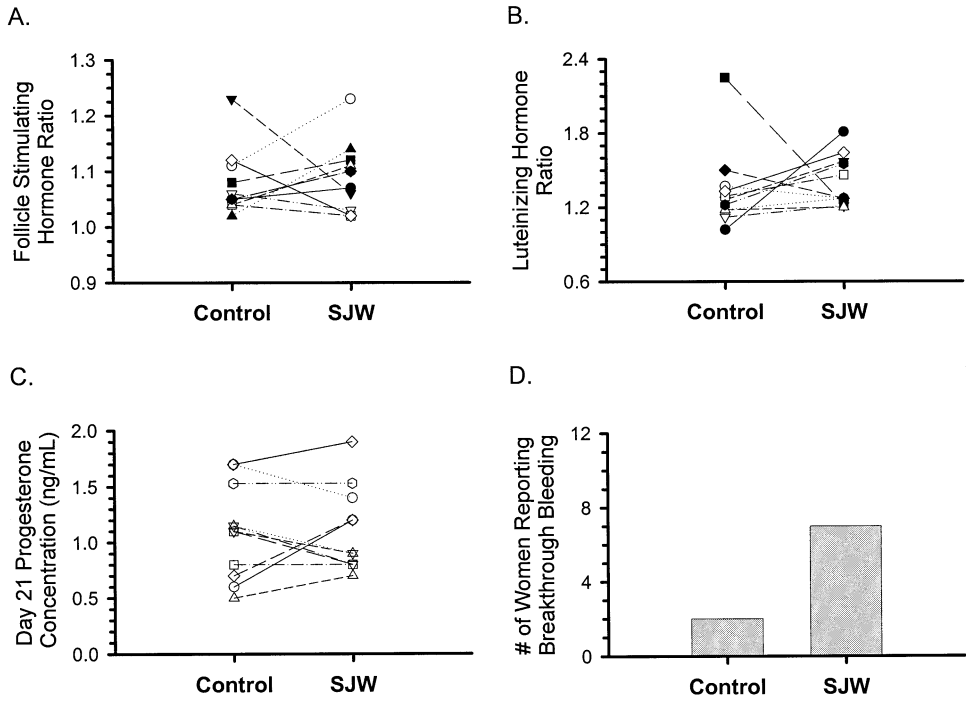

Fig 4. Individual values for ratio of peak to mean serum concentrations of follicle-stimulating hormone (**A**) and luteinizing hormone (**B**) are shown for days 12 through 16 during cycle 1 (control) and cycle 3 (St John's wort [SJW], 300 mg 3 times daily for 8 weeks). Corresponding progesterone serum concentration on day 21 (**C**) and incidence of breakthrough bleeding (**D**) before (control) and after St John's wort administration (300 mg 3 times daily for 8 weeks) are shown in 12 women receiving 1 mg norethindrone and 35 μg ethinyl estradiol.

progestogen ratio resulting in endometrial vascular instability.[24] Although modest changes were seen in the contraceptive hormones in this study, similar changes have been associated with an increased degree of ovarian follicle development.[25]

An interesting observation in this study was the association between oral midazolam clearance in women with breakthrough bleeding (216 ± 67 L/h) compared with the clearance in those women who had no breakthrough bleeding (98 ± 37 L/h) after St John's wort administration. The occurrence of breakthrough bleeding showed a trend toward an association with the extent of norethindrone induction ($CL_{SJW} - CL_{control}$, $P = .059$) and reduced midazolam F_G ($P = .062$). It is unclear as to why we did not observe greater changes in the disposition of norethindrone and ethinyl estradiol, because it is clear from previous investigations that both of these agents are metabolized at least in part by CYP3A.[12,14,26,27]

In this study norethindrone CL_{oral} was increased on average by 16% during St John's wort treatment compared with the control period. This corresponded to a significant reduction in the norethindrone peak concentration but no change in the elimination half-life. These changes are suggestive of an increased first-pass elimination of norethindrone, which is consistent with the reduced bioavailability of the prototypical CYP3A probe midazolam after St John's wort administration.

Midazolam is a well-characterized and widely used selective probe of intestinal and hepatic CYP3A and was used in this study to assess CYP3A modulation by St John's wort. Midazolam CL_{oral} was increased by 50%, whereas F_G showed a concomitant decrease of 50%; F_H and midazolam systemic clearance (intravenous clearance) were unchanged. These findings are consistent with our earlier report and confirm that the effect of St John's wort is largely on intestinal rather than hepatic CYP3A activity.[20] However, the modest changes in norethindrone CL_{oral} in comparison with those seen with oral midazolam clearance after St John's wort dosing suggest that, in addition to CYP3A, other enzymes may play significant roles in the in vivo

disposition of norethindrone. Alternatively, norethindrone may be extensively metabolized by CYP3A enzymes, but first-pass metabolism in the intestinal wall, the major target of St John's wort induction, by CYP3A is not significant.

In contrast to the significant changes in the CL_{oral} of norethindrone and midazolam, we observed a significant change only in the elimination half-life of ethinyl estradiol after St John's wort consumption. The mean CL_{oral} of ethinyl estradiol was increased by 47%, but this change was not significant. However, we did observe marked interindividual variability in the pharmacokinetic parameters of ethinyl estradiol, which is consistent with earlier reports.[28] In previous studies it has been suggested that important individual changes were obscured by the large variability of pooled results and that some individuals may be at higher risk for contraceptive failure than others.[29]

The variable effect of St John's wort dosing on the pharmacokinetic parameters of ethinyl estradiol may reflect the contribution of multiple pathways of metabolism to elimination. The oxidative metabolism of ethinyl estradiol to 2-hydroxy ethinyl estradiol is catalyzed by CYP3A, but this route of elimination accounts for only 30% of the dose.[12,30] In addition, sulfation has been reported to account for 60% of the first-pass elimination of ethinyl estradiol.[13] Glucuronidation also plays an important role in the elimination of ethinyl estradiol, with up to 30% of a dose eliminated in the feces.[31] The glucuronide conjugates of ethinyl estradiol eliminated into the gastrointestinal tract are subject to hydrolysis and subsequent reabsorption of the parent compound (enterohepatic recirculation). Rifampin, a potent inducer of CYP3A, uridine diphosphate–glycosyltransferases, and sulfotransferases, results in significant changes in ethinyl estradiol disposition.[14,26,27] For example, rifampin increased ethinyl estradiol clearance 2-fold, whereas the AUC and half-life were decreased by 66% and 48%, respectively.[14] Likewise, corresponding changes in norethindrone clearance, AUC, and half-life showed a 2-fold increase, a decrease of 51%, and a decrease of 60%, respectively, after rifampin administration. However, the full spectrum of enzyme induction caused by St John's wort is poorly defined, and both the potency and tissue selectivity may contribute to the different effects of St John's wort and rifampin on these compounds.

Hyperforin appears to be the constituent of St John's wort that induces CYP3A through the steroid X receptor pathway.[32] The average steady-state concentrations of hyperforin achieved in this study, 20 ng/mL, is comparable to that from earlier studies.[20,33] However,

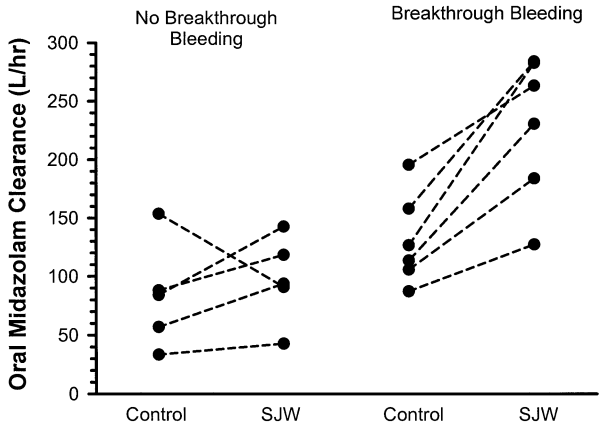

Fig 5. Oral clearance of midazolam before (control) and after St John's wort (SJW) administration (300 mg 3 times daily for 8 weeks) in 7 women who had breakthrough bleeding and 5 women who did not have breakthrough bleeding while receiving 1 mg norethindrone and 35 μg ethinyl estradiol.

serum hyperforin concentrations showed substantial (3-fold) interindividual variability. This variability in serum hyperforin concentrations may also contribute to the observed variability in response to St John's wort.

There were no significant changes observed in the circulating concentrations of FSH, LH in the follicular phase, or progesterone concentration in the luteal phase during St John's wort administration. This result may reflect the modest effect of St John's wort on the pharmacokinetics of ethinyl estradiol and norethindrone. However, the lack of effect of St John's wort on circulating concentrations of FSH, LH, and progesterone is not without precedence. Despite the remarkable effect of rifampin on the pharmacokinetics of ethinyl estradiol and norethindrone and a small but significant effect on FSH concentrations, rifampin administration did not affect circulating LH and progesterone concentrations.[14]

This study was designed to elucidate the mechanism underlying the reports of "miracle babies" in the lay press and of unwanted pregnancy associated with the use of St John's wort in women taking oral contraceptives.[16-18] Despite our failure to find evidence of ovulation and complete loss of oral contraceptive efficacy, we did observe a significant increase in breakthrough bleeding with St John's wort, a finding that is consistent with previous reports. Only modest changes in ethinyl estradiol and norethindrone pharmacokinetics were observed, and because of the substantial interindividual variability, changes in oral contraceptive exposure were not clearly linked to the increased

incidence of breakthrough bleeding. Larger studies are warranted to evaluate the potential of St John's wort to allow ovulation when oral contraceptives are prescribed. Nevertheless, women should be counseled that breakthrough bleeding may occur when these two popular products are coadministered.

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The authors have identified no conflicts of interest in relation to this manuscript.

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